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Clinical Studies in Hemostasis and Thrombosis at Temple University School of Medicine

Mechanism of the Coagulopathy Associated With Acute Promyelocytic Leukemia

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Robert Kaplan, Hematology Fellow, Department of Medicine and Sol Sherry Thrombosis Research Center, Temple University School of Medicine: The patient is a 64-year-old Caucasian female with a history of hypertension and diabetes mellitus who presented with a four-month history of weight loss and fatigue. Laboratory evaluation revealed a pancytopenia initially suspected to be related to her multiple medications. Despite changes in her therapeutic regimen, there was no significant improvement in her blood cell counts. A subsequent bone marrow aspirate revealed atypical promyelocytes with abundant Auer rods accounting for 35% of the marrow cells. Cytogenetic studies detected the t(15,17)(q^{22},q^{21}) translocation in 25 of 27 metaphases counted and the diagnosis of acute promyelocytic leukemia (APL) was confirmed. The patient received standard induction therapy with Idarubicin (12 mg/m^2 iv, three days) and Cytarabine (100 mg/m^2, continuous infusion, seven days). Bone marrow examination at 35 days after beginning therapy documented bone marrow remission. Consolidation treatment consisted of high-dose Cytarabine at a reduced dose (1 gm/m^2 q12 hr × 6 doses) due to her age (>60 years). Due to multiple episodes of bacteremia, consolidation therapy was discontinued after two cycles. A post-consolidation marrow examination confirmed continued remission. Six months after induction, the patient reported easy bruising and increasing fatigue. A complete blood count (CBC) revealed pancytopenia with a white blood count (WBC) of 2,200 per cubic millimeter, hematocrit 21.9%, platelets of 21,000 per cubic millimeter concomitant with a slightly prolonged prothrombin time of 13.6 sec, (normal range, <13.5 sec), a moderately prolonged activated partial thromboplastin time of 39.5 sec (normal range, <35 sec), and decreased fibrinogen of 170 (normal, >200 mg/dl). Fibrinogen degradation products were also found to be elevated at 40 μg/ml (normal range, <10 μg/ml). A peripheral blood smear review showed a moderate number of schistocytes (>5/HPF) The patient was managed with packed red blood cells (PRBC) and platelet transfusions (for active bleeding) and begun on all-*trans*-retinoic acid (ATRA) as salvage therapy. Within a few days, there was normalization of the prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen and by approximately eight weeks, she entered a second clinical remission (confirmed by bone marrow examination). She was able to tolerate ATRA without any major toxicity, and with an alternating two-week on/two week off schedule, she remained free of leukemia with adequate cell counts for another 12 months. Thereafter, she developed generalized weakness and cutaneous and mucous membrane bleeding. A disseminated intravascular coagulation (DIC) panel namely, fibrinogen (150 mg/dl), PT (14.2 sec), aPTT (40 sec), and fibrinogen degradation products (FDP) (>40 μg/ml)) was again markedly abnormal and bone marrow examination confirmed recurrent APL. Supportive care measures were instituted. A few days later she expired secondary to infection.

Raul A. DeLa Cadena, Assistant Professor of Pathology & Laboratory Medicine and Thrombosis Research, Temple University School of Medicine: This case of APL

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is unusual in that the initial presentation was not associated clinically with a coagulopathy. However, the typical balanced reciprocal chromosomal translocation between chromosomes 15 and 17 was identified and eventually clinical and laboratory evidence of a coagulopathy developed. The appearance of the coagulopathy could not be attributed to chemotherapy in this patient since it occurred six months after induction treatment. I would like to ask Dr. Kaplan why this patient was not treated with ATRA initially?

Dr. Kaplan: I cared for this patient in 1993 when ATRA was not yet accepted as standard induction therapy. The patient was considered for an ATRA investigative protocol, but due to her advanced age and comorbidity, she did not fulfill the protocol's criteria.

Dr. DeLa Cadena: Easy bruising is a common clinical finding associated with APL. From the laboratory point of view, although a low platelet count, with a prolongation of both PT and aPTT and low functional levels of fibrinogen are not unique for a specific hemostatic abnormality, they certainly suggest that an excess of thrombin is being generated. DIC, which is a syndrome associated with multiple underlying disorders, could explain the coagulation parameters in this patient. The presence of schistocytes in the peripheral blood smear further supports the diagnosis of DIC. However, APL usually exhibits a coagulopathy combining DIC and excessive fibrinolysis. Thus, since no additional laboratory data is available to confirm generation of either thrombin or plasmin, the coagulopathy can then be attributed to both DIC and primary fibrinolysis. Correction of the coagulopathy by treatment with ATRA is another classic feature of APL found in this case as is the development of resistance to treatment (ATRA) with reappearance of the coagulopathy. Thus, I would like to briefly review the biology and the current knowledge of the coagulopathy associated with APL.

APL is a specific type of acute myeloid leukemia characterized by a distinct morphology of blast cells (M_3) according to the French-American-British (FAB) classification, and the t(15,17)(q^{22},q^{21}) translocation, fusing the promyelocytic leukemia (PML) gene on chromosome 15 to the retinoic acid receptor (RARα) gene on chromosome 17. APL usually exhibits a coagulopathy combining DIC and excessive fibrinolysis. APL is distinguished cytologically by an arrest at the promyelocytic stage of myeloid differentiation. Morphologically, the leukemic cells usually contain a reniform nucleus, large azurophilic granules, and abundant Auer rods [1]. As analyzed by flow cytometry, the promyelocytes are usually $CD34^-$, $CD11b^-$, $CD14^-$, HLA DR^-, and $CD33^+$ [2]. Through the t(15,17)(q^{22},q^{21}) translocation associated with PML, leukemic cells harbor PML/RARα fusion transcripts and to a lesser extent the reciprocal RARα/PML transcripts.

Sunita Sheth, Assistant Professor of Medicine and Thrombosis Research, Temple University School of Medicine: Does the translocation result in a new protein product?

Dr. DeLa Cadena: Yes, transcripts coding for the fusion proteins can now be found in all APL cases by reverse transcriptase-polymerase chain reaction (RT-PCR) and can be used as a tool for molecular diagnosis and detection of minimal residual disease. Other cases of APL have been reported that harbor chromosomal translocations not involving chromosome 15, such as t(11;17) and t(5;17) [3,4].

APL-associated coagulopathy results from at least two distinct mechanisms, namely increased procoagulant activity and fibrinolysis. The most consistently observed laboratory abnormalities include thrombocytopenia, prolongation of the PT, aPTT, and thrombin time, with increased levels of fibrin degradation products and decreased fibrinogen levels. Several types of procoagulant activity have been found in APL cells. A few examples include activation of factor X via intrinsic and extrinsic pathways (including tissue factor-like activity), a unique procoagulant with cysteine proteinase activity (distinct from tissue factor) capable of directly activating factor X [5], and cytokines secreted from leukemic cells capable of inducing procoagulant activity in endothelial cells [5].

Increased fibrinolysis is the second abnormality contributing to the hemostatic defect in APL. Tissue plasminogen activator (t-PA) and single chain prourokinase (u-PA) are released from endothelial cells. Leukemic promyelocytes have been shown to contain uPA and t-PA in sufficient quantities to generate plasmin. Elastase can inactivate α_2-antiplasmin, thus promoting activation of the fibrinolytic system. Decreased plasminogen activator inhibitor-1 (PAI-1) has also been reported in patients with APL.

Therefore, there are multiple mechanisms to explain the coagulopathy observed in APL [5].

Dr. Sheth: Consequently, if there is an increase in fibrinolytic activity due to these enzymes, do you think that the coagulopathy will not to be as amenable to standard therapy for DIC and bleeding, that is, cryoprecipitate and fresh frozen plasma (FFP)?

Dr. DeLa Cadena: That is an interesting question but difficult to answer. This patient, for instance, with bleeding symptoms, was given PRBC and platelets and no FFP, but only treatment with ATRA reversed the coagulopathy. In my view, the increased fibrinolysis and DIC associated with APL is probably different from that encountered in other conditions like systemic inflammatory response syndrome (SIRS). Perhaps others in the audience would like to respond to Dr. Sheth's question.

Robert W. Colman, Sol Sherry Professor of Medicine, Chief, Hematology Section and Director Sol Sherry

Thrombosis Research Center: APL-associated coagulopathy remains a difficult disease to treat.

Ronald Rubin, Professor of Medicine and Thrombosis Research, Temple University School of Medicine: During the late eighties and early nineties, it was reported in the literature that this was a condition to be treated with heparin because its coagulopathy most often could be attributed to DIC. Interpretation of the data was initially difficult because the majority of studies were retrospective, not controlled, and involved a small number of patients. Subsequently, prospective, controlled studies with a sufficient number of patients indicated that heparin did not control the APL-associated coagulopathy [5]. Clinicians thus sought other approaches to control the coagulopathy of APL.

Jay Herman, Professor of Pathology & Laboratory Medicine, Director of Blood Bank, Temple University School of Medicine: Can I ask you about the use of FFP alone in this patient as part of the initial therapy? Would others not advise FFP concomitantly with heparin?

Dr. Kaplan: This remains a continued source of great controversy. I would like to add that today ATRA treatment can effectively and safely reverse the coagulopathy, which in turn may render the potential role of FFP and heparin in this condition obsolete.

Patricia Cohen, Assistant Professor of Pathology & Laboratory Medicine: Is the microgranule variant of APL any different from the point of view of the coagulopathy?

Dr. DeLa Cadena: Not to my knowledge, but I am glad that this case has stimulated an interesting discussion with important questions with regard to the APL-associated coagulopathy and its treatment. In my view, two leading enzymes could account for many of the hemostatic abnormalities associated with APL: plasmin and human neutrophil elastase.

I would like to cite a recent manuscript by Federici, et al. [6]. Plasma von Willebrand factor (vWF) was analyzed in five patients with APL before and after treatment with ATRA. The objective of the study was to evaluate whether vWF structure and function are affected by the proteolytic state found in APL. Proteolysis was investigated with immunopurified vWF from each patient's plasma, which in turn was probed with different monoclonal antibodies directed against vWF by the use of Western-blotting techniques. At diagnosis of APL, a 225 kd native subunit was relatively decreased with appearance of an array of proteolytic fragments, ranging from 140–225 kd. These degradation products were similar to fragments produced in vitro by elastase and plasmin. After ATRA treatment, proteolysis diminished progressively in parallel with improvement of hemostatic parameters. The authors concluded that this mechanism explains in part the clinical bleeding observed in patients afflicted with APL since degraded vWF will not support platelet adhesion to endothelium. Perhaps more interesting is the observation that plasmin and elastase are playing a major role by escaping inhibition from their naturally occurring inhibitors in plasma. In the aforementioned study, ATRA reversed the coagulopathy and resulted in the disappearance of vWF fragments likely generated by elastase and plasmin. This leads me to discuss briefly the mechanism of action of ATRA. Treatment with ATRA leads to modulation of myeloid differentiation associated parameters such as decrease of cathepsin G gene expression, and induction of expression of granulocyte-macrophage colony stimulating factor (GM-CSF) receptors and granulocyte adhesion molecules (CD11b, CD15, CD45RO). These differentiated leukemic cells can no longer produce leukemic clones in soft agar; however, ATRA therapy alone cannot eliminate the leukemic clone. Cells that harbor chromosomal translocations resulting in rearrangements of the RARα gene but involving chromosomes other than chromosome 15 do not differentiate in the presence of ATRA. The effect of ATRA in a cell is mediated by its binding to specific cell retinoic acid binding protein receptors (CRABPs and RARs, RXRs). APL cells do not express detectable CRABPII levels, predominantly express the PML-RARα genes, (and to a lesser extent the RXRα gene) as well as low levels of the remaining normal RARα allele [2]. In the presence of high concentrations of ATRA, PML/RARα can activate RA-inducible reporter genes and restores RA-mediated differentiation in PML/RAR transfected HL-60 cells. Immunostaining with anti-PML antibodies has shown that ATRA treatment relocalizes PML to the normal nuclear structure. This may imply that the PML/RARα protein could be responsible both for the oncogenic effect and the responsiveness of APL cells to ATRA.

A commonly used aphorism in pharmacology is that a drug with no associated side effects is probably not an effective drug. There are associated side effects in approximately 10% of APL patients treated with ATRA. Progressive increase in WBC counts without symptoms is observed with ATRA treatment, but in some cases a rapid increase of WBC counts associated with cardiopulmonary and renal failure has been reported. In 1992, Frankel et al. [7] gave a precise clinical description of a syndrome combining fever, respiratory distress syndrome, weight gain, lower extremity edema, pleural or pericardial effusion, hypotension, and sometimes renal failure. The signs are preceded by increasing WBC in the majority of cases, but some patients develop symptoms at normal WBC counts. The pathophysiology remains poorly understood but because its clinical signs are reminiscent of those observed in adult respiratory distress syndrome (ARDS) and SIRS, a possible stimulatory effect of ATRA on cytokine expression in APL cells has been postulated. Induction of interleukin (IL)-1β and G-

CSF secretion in APL cells under the influence of ATRA may contribute to hyperleukocytosis in vivo. Equally important, secretion of IL-1β, IL-6, TNFα, and IL-8 (which are involved in leukocyte activation and adherence) are implicated in the development of ARDS and SIRS, and could have a pathogenetic role in the ATRA syndrome. Recent studies indicate increased adhesion of NB4 cells (a promyelocytic cell line) to cultured human endothelial cells, endothelial cell matrix, and endothelial cells treated with IL-1 [8].

Dr. Colman: What is the incidence of the ATRA-syndrome; does it occur in every patient?

Dr. DeLa Cadena: No, the ATRA syndrome occurred in about 23% of the patients in the New York experience and it was lower in the French and Japanese experiences. Early addition of chemotherapy in cases with increasing WBC counts may explain the lower incidence in the French and Japanese results [2].

Dr. Colman: Does the occurrence of the ATRA syndrome correlate with the WBC?

Dr. Kaplan: Although the ATRA syndrome occurs more frequently in patients with preceding leukocytosis while on treatment, there are certainly patients who develop the syndrome with normal WBC counts, as well as patients with striking leukocytosis who never develop symptoms of the syndrome at all. In one study of 78 patients, the occurrence of the syndrome was positively associated with the peak value of the peripheral blood leukocyte count after ATRA therapy had been initiated [9]. In addition, no correlation of the occurrence of the ATRA syndrome has been made with initial WBC at diagnosis.

Dr. Colman: What is the current status with regard to the cysteine protease that can directly activate factor X? I have never been sure of its role. Do you know whether the enzyme has been cloned?

Dr. DeLa Cadena: I have not seen any further information in the literature. I mentioned it because, until it is proved to be irrelevant, it remains one of the potential mechanisms to explain the APL-associated coagulopathy.

Finally, I point out that resistance to ATRA has limited the progress in the treatment of APL. The mechanism of action accounting for resistance to ATRA therapy relates to increased hepatic enzymes (cytochrome P450 system) involved in ATRA metabolism [2]. Thus, until superior treatment strategies are developed, regulation of plasmin and elastase by specific protease inhibitors may represent an alternative therapeutic modality capable of preventing the coagulopathy characteristic of APL.

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